FEBS Letters 583 (2009) 3412–3418

journal homepage: www.FEBSLetters.org

Δ^9-Tetrahydrocannabinol regulates the p53 post-translational modifiers Murine double minute 2 and the Small Ubiquitin MOdifier protein in the rat brain

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ARTICLE INFO

Article history:
Received 6 August 2009
Revised 22 September 2009
Accepted 30 September 2009
Available online 9 October 2009

Edited by Varda Rotter

Keywords:
Cannabinoid
Δ^9-Tetrahydrocannabinol
Murine double minute
Small Ubquitin-like MOdifier
Post-translational modification
Signal transduction

ABSTRACT

The phytocannabinoid Δ^9-Tetrahydrocannabinol (Δ^9-THC), the main psychoactive cannabinoid in cannabis, activates a number of signalling cascades including p53. This study examines the role of Δ^9-THC in regulating the p53 post-translational modifier proteins, Murine double minute (Mdm2) and Small Ubquitin-like MOdifier protein 1 (SUMO-1) in cortical neurons. Δ^9-THC increased both Mdm2 and SUMO-1 protein expression and induced the deSUMOylation of p53 in a cannabinoid receptor type 1 (CB_1)-receptor dependent manner. We demonstrate that Δ^9-THC decreased the SUMOylation of the CB_1 receptor. The data reveal a novel role for cannabinoid receptor activation in modulating the SUMO regulatory system.

Structured summary:
MINT-7266621: *Cb1* (uniprotkb:P20272) *physically interacts* (MI:0915) with *SUMO-1* (uniprotkb:Q5I0H3) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-7266633: *SUMO-1* (uniprotkb:Q5I0H3) and *Cb1* (uniprotkb:P20272) *colocalize* (MI:0403) by *fluorescence microscopy* (MI:0416)
MINT-7266611: *p53* (uniprotkb:P10361) *physically interacts* (MI:0915) with *SUMO-1* (uniprotkb:Q5I0H3) by *anti bait coimmunoprecipitation* (MI:0006)

1. Introduction

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the main psychoactive component of *Cannabis sativa* [1–3]. Δ^9-THC activates the cannabinoid receptor type 1 (CB_1) which is expressed in the central nervous system. CB_1 is a G-protein coupled receptor that regulates ion channels and activates multiple signal transduction mechanisms [4]. The ability of cannabinoids to promote cell death and cell survival is an intriguing aspect of cannabinoid action [5]. Cannabinoid-induced neurotoxicity has been reported in neurones [6–9] and in the neonatal cerebral cortex [10]. Contrary to this, Δ^9-THC has neuroprotective effects [11].

The tumour suppressor protein, p53, has a central role in cell cycle arrest or apoptosis in response to a variety of stressors [12,13]. The activity of p53 can be titrated at many levels including transcriptional control, alterations in protein half-life and post-translational modifications [14]. Post-translational modifications of p53 allow cells to rapidly activate a unique set of responses to diverse stress stimuli [15]. In unstressed cells, p53 is present in a latent state and is maintained at low levels through targeted degradation by association with Murine double minute 2 (Mdm2) [16]. The pro-apoptotic function of p53 is induced by phosphorylation of serine 15 which we have previously reported to occur in neurones treated with Δ^9-THC [7,8]. Phosphorylation of serine 15 also inhibits the interaction between p53 and Mdm2 [17].

The post-translational modification of p53 with the Small Ubiquitin-related MOdifier protein 1 (SUMO-1) affects the activity of p53 [18,19]. SUMO-1 binds covalently to lysine residues located within a SUMOylation consensus motif located in multiple substrate proteins [20]. SUMO-1 binds to the C-terminus of p53 within the SUMO conjugation motif (ΨKXE) [21]. Contrary to reports of the positive effects of SUMOylation on p53 transactivation [18,19], it has been demonstrated that SUMOylation of p53 decreases the strength of the interaction between p53 and Mdm2 [22]. Although p53 was one of the first proteins that SUMOylation was demonstrated, the physiological relevance of this modification remains enigmatic [21]. Protein SUMOylation has a role in a num-

Abbreviations: Δ^9-THC, Δ^9-Tetrahydrocannabinol; CB_1, the cannabinoid receptor type 1; Mdm2, Murine double minute 2; SUMO-1, Small Ubquitin MOdifier protein 1; Ψ, large hydrophobic residue; K, lysine; X, any amino acid; E, glutamic acid; HRP, horse radish peroxidase; WB, Western immunoblot; IP, immunoprecipitation

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0014-5793/$36.00
doi:10.1016/j.febslet.2009.09.056

ber of physiological processes, including nuclear transport [23], cell cycle control [24] and synaptic transmission [25]. Furthermore, recent reports have implicated the SUMO regulatory processes in neurodegeneration [26]. Given the role for cannabinoids in controlling neuronal fate and synaptic function [27,28] we examined whether activation of the cannabinoid system by Δ^9-THC affected the expression of Mdm2 or SUMO-1 in primary neurones. Furthermore, we investigated if the SUMOylation status of p53 and CB_1 were altered by Δ^9-THC.

2. Methods

2.1. Culture of cortical neurones

Primary neurones were established as previously described [7,8].

2.2. Drug treatments

Δ^9-THC was obtained from Sigma–Aldrich Company Ltd. and diluted to 5 μM with culture media [7,8]. Neurones were incubated with the selective CB_1 antagonist AM251 (10 μM; [7,8,29], Tocris Cookson Ltd.) for 30 min before Δ^9-THC treatment.

2.3. Western immunoblot

Neurones were harvested in lysis buffer as previously described [7,8] and supplemented with NP-40 (0.5%) and SDS (10%) for determination of SUMO-1 expression. Proteins were separated by electrophoresis, transferred to nitrocellulose and detected with specific antibodies for Mdm2 (1:500; Abcam) or SUMO-1 (1:500; Abcam) followed by horse radish peroxidase (HRP)-conjugated anti-rabbit IgG (1:500/1:800) and enhanced chemiluminescence. Blots were exposed to X-ray film followed by imaging and quantification using Lab works image acquisition and analysis software (UVP Bioimaging systems). Blots were stripped and re-probed for β actin. Data are expressed as ratios of target protein/β actin using arbitrary units. Unknown molecular weights were read from a standard curve plotted from the distances travelled by molecular weight markers against their molecular weights (250–7 kDa).

2.4. Immunocytochemistry

Neurones were fixed with 4% paraformaldehyde, permeabilised with 0.2% Triton X-100 and blocked in PBS containing 20% horse or 10% goat serum for Mdm2/SUMO-1, respectively. Proteins were labelled with specific antibodies for Mdm2 (1:800; Chemicon) or SUMO-1 (1:100; Santa Cruz Biotechnology Inc.) followed by biotinylated goat anti-mouse IgG (1:500/1:200) and avidin-conjugated ALEXA488® (1:50). Incorporated fluorophores were examined by confocal microscopy using appropriate excitation wavelengths and filters.

2.5. Immunoprecipitation

Protein was prepared (600 μg/ml) as previously described [30] was incubated with 5 μg of goat anti-total p53 antibody or goat anti-CB_1 (N19/N15, respectively; Santa Cruz Biotechnology Inc.). Immune complexes were obtained by adding 50 μl TrueBlot™anti-goat IgG beads (eBiosciences). Bound protein was eluted by heating samples in sample buffer, separated by electrophoresis, transferred to nitrocellulose and blocked in 5% non-fat milk (NFM) in buffer A (25 mM Tris–HCl, pH 7.3, 0.15 M NaCl, 0.1% Tween-20). Blots were probed for SUMO-1 (1:400; Abcam), total p53 (1:500; R19; Santa Cruz Biotechnology Inc.) or CB_1 (1:1000; N15; Santa Cruz Biotechnology Inc.) followed by detection with HRP-conjugated secondary IgG (1:400; 1:1800; 1:1500). All dilutions were with 0.5% NFM in buffer A. Immunoreactive bands were detected and quantified as in Section 2.3.

2.6. Colocalisation of CB_1/SUMO-1

Following SUMO-1 labelling (Section 2.4) CB_1 was labelled with goat anti-CB_1 (1:500 in 10% horse serum; N15; Santa Cruz Biotechnology Inc.) followed by biotinylated anti-goat IgG (1:500 in 10% horse serum) and ExtrAvidin®-R-Phycoerythrin (1:50). Coverslips analysed as in Section 2.4.

2.7. In silico protein SUMOylation site prediction

CB_1 protein sequence (GenBank: AAA99067.1) was searched for potential SUMOylation sites using two algorithms: SUMOplot™ (http://www.abgent.com/tools/sumoplot) and SUMOsp v2.0 (http://sumosp.biocuckoo.org/) [31] using a medium threshold level for SUMOsp v2.0.

2.8. Statistics

Data are reported as the means ± S.E.M. of the number of experiments indicated in each case. For comparisons between relevant treatments an un-paired Student's *t*-test was performed using Graph Pad Prism 5.

3. Results

3.1. Δ^9-THC evokes an increase in Mdm2

The effect of Δ^9-THC treatment (5 μM; 5–120 min) on Mdm2 expression was assessed (Fig. 1). The Mdm2 antibody was immunoreactive against (i) a 72 kDa isoform of Mdm2 generated from one of the p53 response elements, (ii) a 60 kDa isoform corresponding to the cleavage product generated from the full length (90 kDa) Mdm2 and (iii) a 30 kDa C-terminal fragment of the Mdm2 (Fig. 1B). Δ^9-THC treatment only affected the expression of p72Mdm2. Following Δ^9-THC treatment for 5 min, p72Mdm2 expression was 2.33 ± 0.28 arbitrary units (mean ± SEM) which was significantly higher than in neurones treated with vehicle (0.97 ± 0.33; $P < 0.05$, Student's *t*-test, $n = 6$). This was not maintained at subsequent time points (15–120 min; Fig. 1A). Immunocytochemistry demonstrated that Mdm2 was expressed after 5 min treatment with Δ^9-THC and was localised within the cytosol (Fig. 1C). We also observed that this response was CB_1 dependent (Fig. 1B, ii and Fig. 1C, v and vi).

3.2. Δ^9-THC evokes an increase in unconjugated SUMO-1

To further establish the effect of Δ^9-THC on the post-translational modifiers of p53, SUMO-1 expression was also assessed following treatment with Δ^9-THC (5 μM; 5–120 min; Fig. 2A and B). Δ^9-THC evoked a time-dependent increase in the expression of unconjugated SUMO-1. In neurones exposed to Δ^9-THC for 5 min, unconjugated SUMO-1 expression was 104.90 ± 17.07 arbitrary units (mean ± SEM), comparable to neurones treated with vehicle (81.27 ± 12.39). Following treatment with Δ^9-THC for 15 min unconjugated SUMO-1 expression was significantly increased to 134.70 ± 17.49 ($P < 0.05$ vs. vehicle, Student's *t*-test, $n = 4$). This effect was maintained after 30 min treatment with Δ^9-THC (126.20 ± 11.17; $P < 0.05$ vs. vehicle, Student's *t*-test, $n = 4$). This was not maintained at subsequent time points (60 and 120 min; Fig. 2A). Fig. 2A also demonstrates that Δ^9-THC increases unconju-

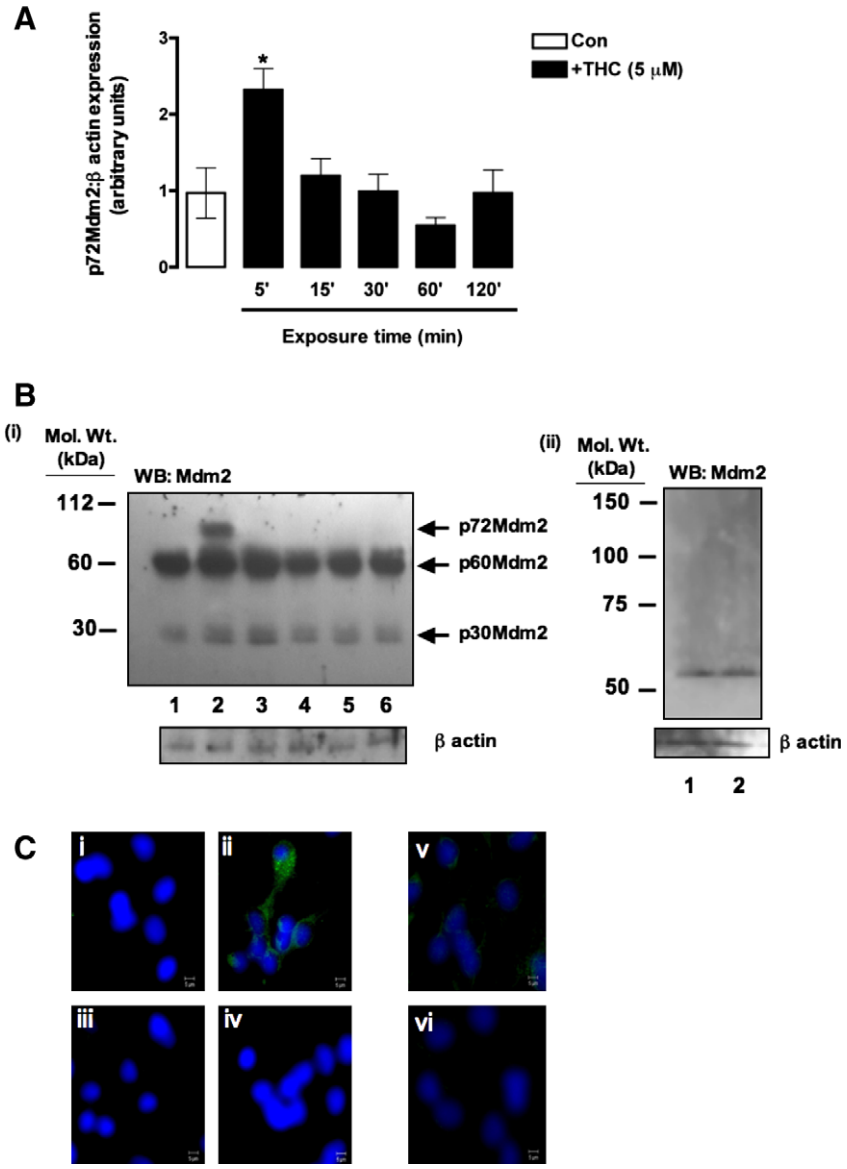

Fig. 1. Δ^9-THC increases p72Mdm2. (A) a significant increase in p72Mdm2 was found following treatment with Δ^9-THC for 5 min and not at other time points (15–120 min). Results are expressed as means ± S.E.M., $^*P < 0.05$ vs. vehicle, Student's *t*-test, $n = 6$. (B) (i) Representative blot demonstrating the induction of p72Mdm2 (arrow ≈ 72 kDa) expression at 5 min (lane 2). Lane 1 represents vehicle-treated neurones, neurones treated with Δ^9-THC for 15 min (lane 3), 30 min (lane 4), 60 min (lane 5) and neurones treated with Δ^9-THC for 120 min (lane 6). The p60Mdm2 cleavage product and C terminal fragment (p30) were also visible on the blot. β Actin expression was monitored to ensure equal protein loading (bottom inset). (ii) Representative blot demonstrates that AM251 prevented the Δ^9-THC-induced expression of p72Mdm2. p72Mdm2 is not expressed in neurones treated with AM251 or Δ^9-THC and AM251 (lanes 1 and 2; compared to lane 2 in B i). (C) Representative overlay images of Mdm2 immunoreactivity (green) in neurones treated with (i) vehicle, and neurones exposed to Δ^9-THC for (ii) 5 (iii) 15 and (iv) 30 min. Also neurones treated with AM251 (v), and neurones exposed to AM251 and Δ^9-THC (5 min, vi). Nuclei were stained with hoechst.

gated SUMO-1 expression via CB_1 since AM251 abrogated the Δ^9-THC-mediated increase in unconjugated SUMO-1 expression. Conjugated SUMO-1 indicated by the presence of a high molecular weight smear was also increased (Fig. 2B, ii) reflective of Δ^9-THC-induced alterations in the overall SUMOylation status of proteins. Immunocytochemistry revealed that SUMO-1 expression was localised in the cytosol and nucleus in Δ^9-THC-treated neurones (Fig. 2C, iii).

3.3. Δ^9-THC induces the deSUMOylation of p53

Since SUMO-1 is a post-translational modifier of p53, linked to p53 transactivation, we assessed if Δ^9-THC regulated the SUMOylation status of p53. Immunoprecipitation was carried out to determine if Δ^9-THC-induced the deSUMOylation of p53 (Fig. 3). Neurones were treated with Δ^9-THC for 15 min in the presence or absence of CB_1 antagonist, AM251. Δ^9-THC treatment for 15 min induced a significant decrease in SUMOylated p53 levels from 2551 ± 312 arbitrary units (mean ± SEM) in vehicle-treated neurones to 1292 ± 145 following a 15 min exposure to Δ^9-THC ($P < 0.01$ vs. vehicle, Student's *t*-test, $n = 6$). AM251 treatment alone had no effect on SUMOylated p53 levels (2902 ± 295), however it prevented the Δ^9-THC-induced decrease in SUMOylated p53 levels (2307 ± 306; $P < 0.05$ vs. Δ^9-THC, Student's *t*-test, $n = 6$). Δ^9-THC induced an increase in total p53 at 5 min (Fig. 3C, lane 2) compared to control neurones (lane 1). The presence of bands greater than 53 kDa in control neurones are indicative of p53 conjugated to interacting proteins, one of which is SUMO-1 (Fig. 3B). These bands were not present in neurones treated with Δ^9-THC for 5, 15 or 30 min (lanes 2, 3, and 4).

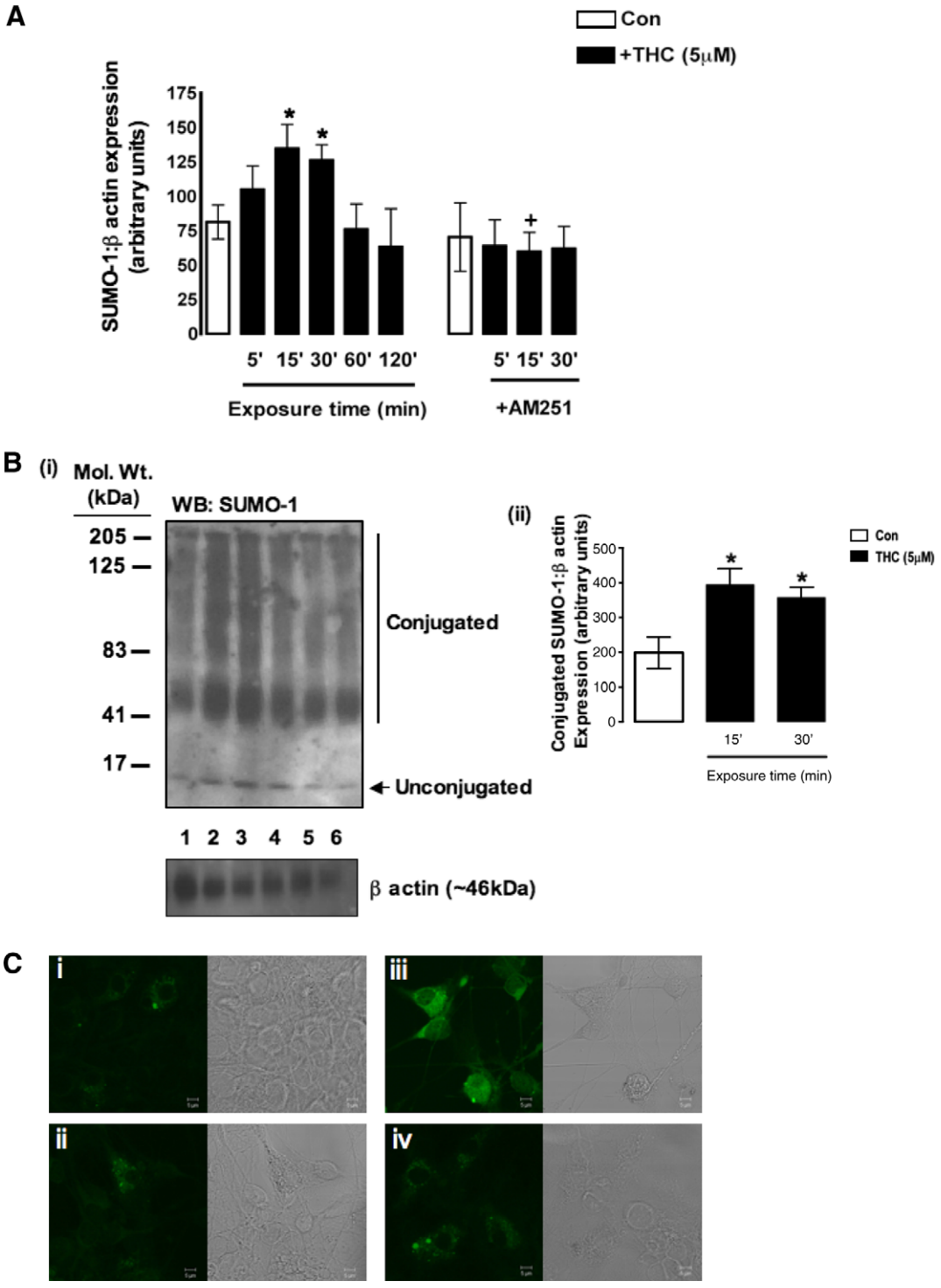

Fig. 2. Δ^9-THC increases SUMO-1. (A) Δ^9-THC significantly increased unconjugated and conjugated SUMO-1 expression at 15 and 30 min but not at other time points (5, 60 and 120 min). Results are expressed as means ± S.E.M., $^*P < 0.05$ vs. vehicle, Student's *t*-test, $n = 4$. The Δ^9-THC-induced increase in unconjugated SUMO-1 expression was abrogated by CB_1 antagonist AM251 ($^+P < 0.05$ vs. Δ^9-THC 15 min, Student's *t*-test, $n = 4$). (B) (i) Representative blot showing the increase in unconjugated and conjugated SUMO-1 expression in neurones exposed to Δ^9-THC for 15 (lane 3) and 30 min (lane 4) compared to vehicle (lane 1) and neurones exposed to Δ^9-THC for 5, 60 and 120 min (lanes 2, 5 and 6). β Actin expression was monitored to ensure equal protein loading (bottom panel). (ii) Δ^9-THC significantly increases conjugated SUMO-1 after 15 and 30 min. Results are expressed as means ± S.E.M., $^*P < 0.05$ vs. vehicle, Student's *t*-test, $n = 4$. (C) Representative confocal images of SUMO-1 expression in neurones treated with (i) vehicle, (ii) Δ^9-THC for 5, (iii) 15 and (iv) 30 min. Differential interference contrast (DIC) images showing the total number of neurones present are also shown.

3.4. SUMOylation status of CB_1

Since Δ^9-THC induces the deSUMOylation of p53 through CB_1 and the publication of several reports showing agonist-induced alterations in the SUMOylation status of many neuronal signalling receptors, we investigated if CB_1 contained potential SUMOylation motifs and if the SUMOylation status of CB_1 was altered by Δ^9-THC. In silico analysis of CB_1 amino acid sequence revealed two potential SUMOlyation motifs (Fig. 4A). SUMOplot™ revealed two highly probable motifs at lysine 43 (K43) and lysine 193 (K193; Fig. 4A, i).

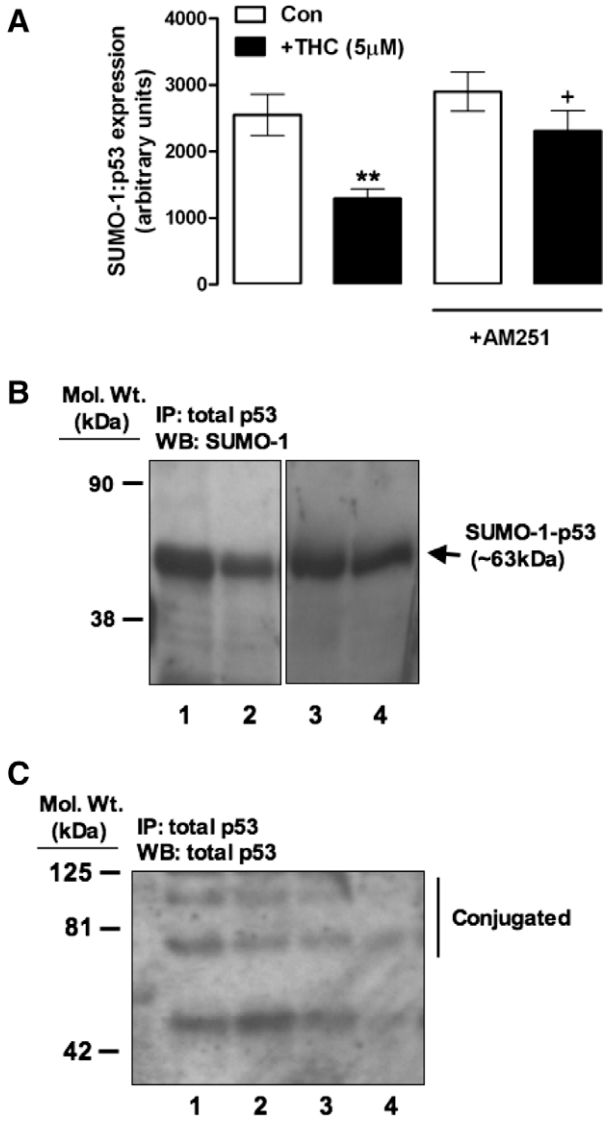

Fig. 3. The effect of Δ^9-THC on p53/SUMO-1 colocalisation. (A) Treatment with Δ^9-THC for 15 min induced a significant decrease in SUMOylated p53. Pre-incubation with AM251 prior to Δ^9-THC exposure abolished the Δ^9-THC-induced decrease in SUMOylated p53 expression. Results are expressed as means ± S.E.M., $^{**}P < 0.01$ vs. vehicle, $^{+}P < 0.05$ vs. Δ^9-THC 15 min, Student's *t*-test, $n = 6$). (B) Representative blot demonstrating the decrease in SUMOylated p53 expression after 15 min of Δ^9-THC treatment (lane 2) compared to control neurones (lane 1). While AM251 had no effect on SUMOylated p53 expression (lane 3), it abolished the ability of Δ^9-THC to decrease SUMOylated p53 expression (lane 4). (C) Representative blot demonstrating total p53 expression in the lysates. Total p53 was increased following 5 min of Δ^9-THC treatment (lane 2) compared to control neurones (lane 1). Bands greater than 53 kDa were present in control neurones but not in neurones treated with Δ^9-THC for 5, 15 or 30 min (lanes 2, 3, and 4).

SUMOsp v2.0 revealed only one motif also at K43 (Fig. 4A, ii) corroborating results achieved with SUMOplot™. In control neurones, immunocytochemistry demonstrated that SUMO-1 and CB_1 were colocalised within the perinuclear area and along neuronal processes (Fig. 4B). In neurones treated with Δ^9-THC (Fig. 4C) SUMO-1 expression was increased whilst a marked internalisation of CB_1 was observed. Colocalisation between CB_1 and SUMO-1 was only observed in vehicle-treated neurones (Fig. 4B, iv) indicating that CB_1 is SUMOylated in the basal state and becomes deSUMOylated upon activation by Δ^9-THC. Immunoprecipitation of CB_1 was performed to validate this result (Fig. 4D, i). In control neurones immunoreactive bands for SUMO-1 and CB_1 were observed (Fig. 4D, ii and iii, lane 1). In neurones treated with Δ^9-THC we only observed bands for CB_1 (Fig. 4D, iii, lane 2) and not SUMO-1 (Fig. 4D, ii, lane 2) indicating that Δ^9-THC induces the deSUMOylation of CB_1.

4. Discussion

We examined the effect of Δ^9-THC on the p53 post-translational modifiers Mdm2 and SUMO-1. Δ^9-THC induced an increase in the expression of the p53-responsive protein, Mdm2, after 5 min coinciding with the increase in phospho-p53^{ser15} which we have previously reported [7]. p53 acts as a transcription factor for Mdm2, thus when activated, p53 transcriptionally upregulates Mdm2 expression [32]. Due to the ability of Mdm2 to inhibit p53, a negative feedback loop is formed providing a tight regulatory mechanism [32]. We observed three isoforms of Mdm2, p72, p60 and p30. This is unsurprising since the Mdm2 gene has two separate p53 response elements each generating different Mdm2 isoforms (p90 and p72). Additionally, proteases can generate shorter Mdm2 isoforms (p60 and p30) [33,34]. The full length Mdm2 isoform (p90Mdm2) is capable of binding and labelling p53 for degradation whilst p72Mdm2 lacks a p53 binding site and so cannot act as a p53 repressor [33]. This study demonstrates that Δ^9-THC increases the expression of the p72Mdm2, effectively activating p53 by preventing its degradation [33]. This may account for the Δ^9-THC-mediated upregulation in p53 expression which we have previously reported to be essential for Δ^9-THC-induced apoptosis [7,8]. This effect of Δ^9-THC could also reflect a transient stabilisation or neo-synthesis of this isoform. The p60Mdm2 and p30Mdm2 isoforms that we observed are probably produced by the action of a caspase-like enzyme, which is normally expressed at high levels in cultured cells [35]. Our findings regarding the induction of the Mdm2 protein provides corroborating evidence that Δ^9-THC modulates proteins that impact on p53 signalling. In addition the observation that the p72Mdm2, which is incapable of degrading p53, was upregulated provides a mechanism that may allow for the ability of Δ^9-THC to stabilise p53 during neuronal apoptosis [7,8].

Δ^9-THC significantly increased the level of unconjugated and conjugated SUMO-1 protein in a time-dependent manner with a maximal response observed after 15–30 min of Δ^9-THC treatment. Δ^9-THC induced the deSUMOylation of p53 which could be responsible in part, for the aforementioned increase in overall unconjugated SUMO-1. AM251, ablated the effect of Δ^9-THC on the SUMOylation status of p53, indicating that deSUMOylation of p53 involved CB_1. The association of p53 with SUMO-1 is linked to the regulation of the transcriptional activity [18,19], intracellular location [22] and the apoptotic potential of p53 [36]. However, there have been conflicting reports as to the functional significance of this p53 modification [21,37]. Its relevance has been questioned since most SUMO targets, including p53, are modified at low levels leaving a limited pool of unconjugated SUMO-1 [20,38]. However, the role of modulating the SUMOylation status of p53 is perhaps a more subtle modification possibly acting in conjunction to other post-translational modifications, e.g., interaction with other p53 interacting proteins. Our observations regarding increased levels of conjugated SUMO-1 following Δ^9-THC treatment may be reflective of this. Protein phosphorylation can act as a positive or negative signal for SUMOylation, e.g., the phosphorylation of c-Jun and p53 induces their deSUMOylation [36,39]. These studies are of particular interest considering our previous findings regarding the Δ^9-THC-induced phosphorylation of p53 at serine 15 [7].

Cytoplasmic, nuclear and plasma membrane associated proteins are modified by SUMO [40–42]. SUMOylation of the transcription factor myocyte enhancer factor 2A plays a role in the neuronal differentiation [43]. We have shown that Δ^9-THC induces

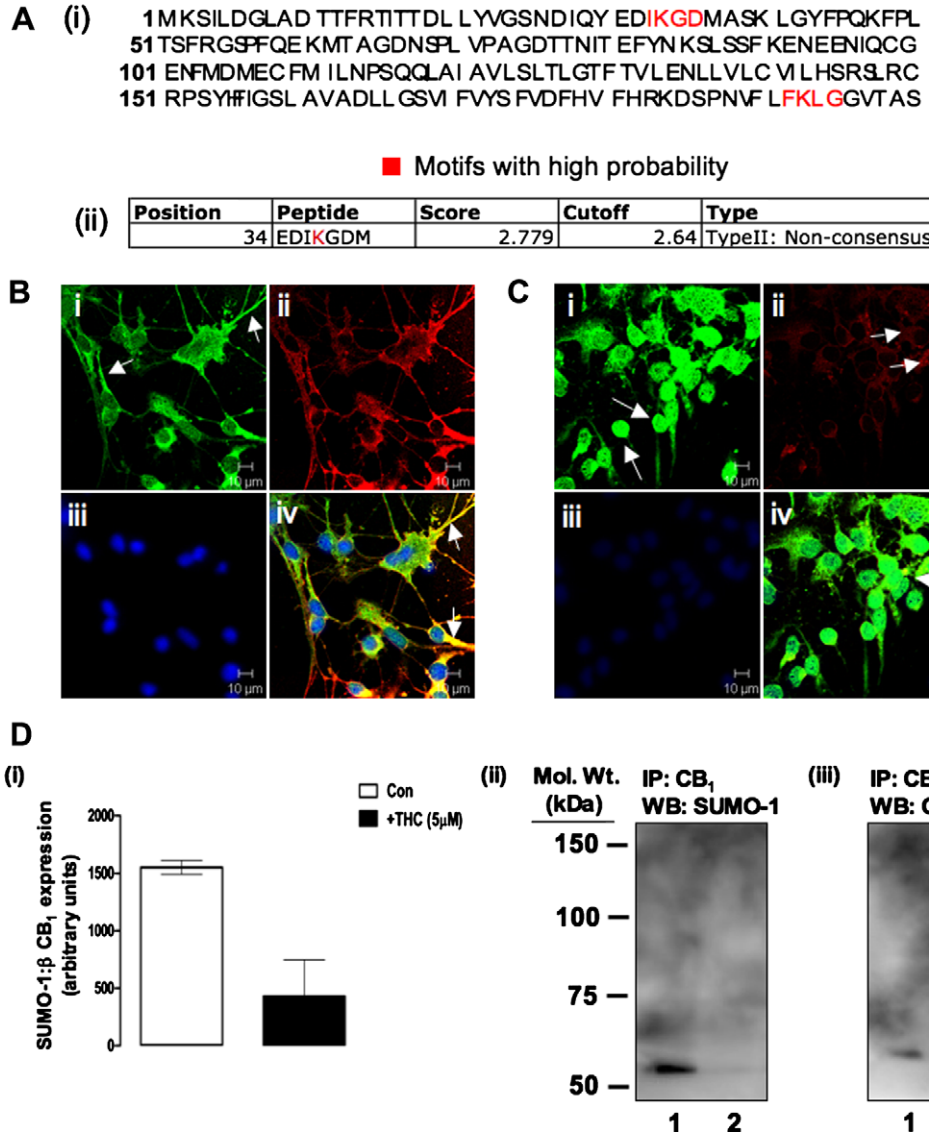

Fig. 4. The effect of Δ^9-THC on CB_1/SUMO-1 colocalisation. (A) Potential SUMOylation motifs found after in silco experiments using SUMOplot™ (i) and SUMOsp v2.0 (ii) SUMOylation motif prediction alogrithms. (B) Representative images of fluorescently labelled SUMO-1 (green; panel i) and CB_1 (red; panel ii) in neurones treated with vehicle. There was a marked colocalisation (yellow; panel iv) between SUMO-1 and CB_1 on neuronal processes and around the cell body (arrows). Nuclei were stained with hoechst (panel iii). (C) Representative images of fluorescently labelled SUMO-1 (green; panel i) and CB_1 (red; panel ii) in neurones treated with Δ^9-THC for 15 min. Treatment with Δ^9-THC caused an overall increase in SUMO-1 and CB_1 internalisation. There was a noticeable reduction in the amount of colocalisation between SUMO-1 and CB_1 (panel iv, yellow, arrows). Nuclei were stained with hoechst (panel iii). (D) (i) Treatment with Δ^9-THC for 15 min decreased SUMOylated CB_1. Results are expressed as means ± S.E.M. of three observations. (ii) Representative blot demonstrating the presence of SUMO-1 in CB_1 immunoprecipitates of control neurones (lane 1) and its absence in Δ^9-THC treated neurones (lane 2). (iii) Representative blot confirming the presence of CB_1 in the immunoprecipitates of control neurones (lane 1) and Δ^9-THC treated neurones (lane 2).

the deSUMOylation of p53 indicating that SUMOylated p53 may be a pro-survival signal in neurones. Gibb et al. found that SUMOylation of the astroglial glutamate transporter EAAT2 causes the redirection of the transporter to the nucleus which is responsible for the transcriptional regulation of genes effecting astrocyte physiology [44]. It has also been suggested that in neurones, SUMOylation permits a rapid and reversible modification of kainite receptor membrane localisation and synaptic transmission [26]. Given that CB_1 is required for Δ^9-THC-induced neuronal signalling and p53 SUMOylation, we also examined whether CB_1 itself was a target for SUMOylation. We identified potential SUMOylation motifs in CB_1 amino acid sequence. The motif involving K43 is highly likely to be a SUMOylation motif due to the high accuracy of the SUMOsp v2.0 algorithm [31]. It would be important to determine the importance of this residue by performing experiments using cells expressing K43 deficient CB_1. Immunocytochemistry and immunoprecipitation (IP) demonstrated that SUMO-1 and CB_1 were colocalised, indicating that under basal conditions CB_1 is SUMOylated. Δ^9-THC induced a marked decrease in the colocalisation between SUMO-1 and CB_1 indicating that activation of CB_1 induces its deSUMOylation or the SUMOylation of a CB_1 receptor-associated protein.

Our findings raise the novel prospect that Δ^9-THC can regulate the SUMOylation of p53 and CB_1 (see overview in Fig. 5). This is of importance considering both phytocannabinoids and SUMO regulatory systems have been implicated in neurophysiological events such as synaptic formation and function, regulation of receptor numbers and neurodegeneration [26,44].

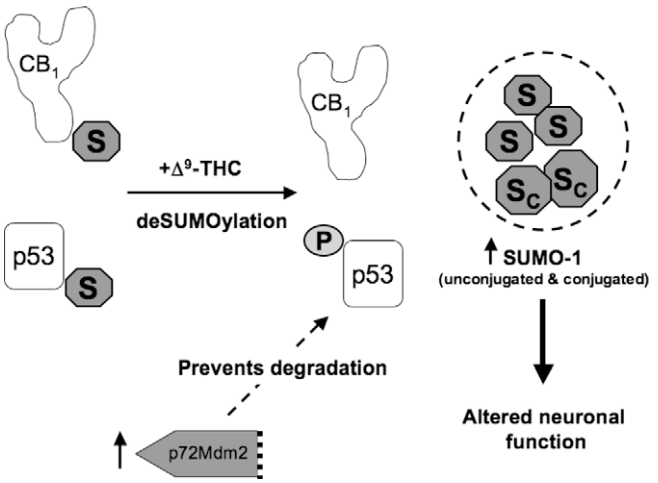

Fig. 5. Schematic representation of the effect Δ^9-THC has on the post-translational modifying proteins Mdm2 and SUMO-1. Δ^9-THC induced an increase in the p72Mdm2 which is incapable of labelling p53 for degradation. Δ^9-THC also induced an increase in unconjugated (S) and conjugated SUMO-1 (Sc) in a CB_1 dependent manner. CB_1 becomes deSUMOylated upon treatment with Δ^9-THC.

Acknowledgments

We thank all reviewers of this manuscript. Funded by Science Foundation Ireland.

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